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Identification of 3q oncogene *SEC62* as a marker for distant metastasis and poor clinical outcome in invasive ductal breast cancer

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Abstract

Purpose In previous studies, we have shown that SEC62 has an essential function in cell migration, epithelial-to-mesenchymal transition, and endoplasmic reticulum stress tolerance of cancer cells. *SEC62* expression correlated with distant and lymph node metastasis and poor outcome in different cancer entities. In this initial study, we investigated *SEC62* expression and its possible role as a prognostic and predictive biomarker in breast cancer (BC).

Methods Formalin-fixed, paraffin-embedded tissue samples of 53 BC patients were analyzed by immunohistochemistry. The immunoreactive score (IRS) according to Remmele and Stegner was evaluated and correlated with clinico-pathological findings and overall survival (OS).

Results We found increased SEC62 protein levels in tumor tissue compared to tumor-free tissue samples from the same patients. Tumors with high *SEC62* expression (IRS > 8), or containing isolated cells with high SEC62 staining intensity, independent of the IRS, had more frequently distant metastases (48.4% vs. 18.2%; $p=0.024$ and 47.4 vs. 6.7%; $p=0.005$, respectively). Overall survival was significantly worse in BC patients with high *SEC62* expression (SEC62 IRS > 8) (54.8% vs. 81.8%; $p=0.011$) and in cases with isolated high-intensity SEC62 staining cells independently of SEC62 IRS (55.3% vs 93.3%; $p=0.024$).

Conclusions We are the first to describe the *SEC62* expression and its correlation to clinicopathological parameters in mammary carcinoma. Our results suggest that *SEC62* expression may serve as a prognostic marker for patients with invasive ductal breast cancer.

Keywords SEC62 · Immunohistochemistry · Breast cancer · Prognostic factor · Biomarker · Metastasis

Introduction

Breast cancer (BC) is the most common cancer overall and one of the main causes of cancer deaths in women worldwide [1]. Despite advances in early detection, prognosis and therapy, mortality remains high [1]. Numerous studies on gene expression have shown that breast cancer is a clinically and molecularly heterogeneous disease whose subtypes have different prognoses and therapeutic responses. Through the establishment of various factors, such as estrogen and progesterone receptor and human epidermal growth factor receptor 2 (HER2) status, BC prognosis may be assessed with increasing accuracy [2–4]. A small number of expression profiling strategies have been successfully developed and validated for clinical use. This improves the decision-making process with regard to the choice of therapy and enables individualized therapy [5–9]. However, further prognostic markers are needed to better adapt the treatment strategy to the disease.

SEC62, a gene located at chromosomal region 3q26.2 that encodes an endoplasmic reticulum transmembrane protein, is overexpressed in various tumor types. Elevated mRNA concentrations were observed in several studies in tumor cells [10, 11]. Further studies showed higher SEC62 protein content in several cancer cell lines and in cancer tissue

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samples [10–13]. Linxweiler et al. have found a statistically significant correlation between elevated SEC62 protein levels and lymph node metastasis, as well as a significantly worse overall survival (OS) in non-small cell lung cancer (NSCLC) cases [10]. In thyroid cancer, the increased SEC62 protein content was found to correlate with advanced disease, including lymph node metastases, invasion of lymph and blood vessels, or excessive infiltration of the thyroid capsule [10]. Wemmert et al. have observed a significantly poorer OS and progression-free survival (PFS) in head and neck squamous cell carcinoma (HNSCC) in cases with high SEC62 protein levels [13]. Moreover, high *SEC62* expression levels have been found in lymph node metastases from HNSCC patients and patients with CUP syndrome (cancer of unknown primary) and are also associated with a significantly worse prognosis [14]. In previous studies, we have shown that SEC62 has an essential function in cell migration, epithelial-to-mesenchymal transition (EMT), and endoplasmic reticulum stress tolerance of cancer cells [10, 12, 15]. 3q amplification and, in particular, *SEC62* overexpression could also be detected in BC [12, 16]. *SEC62* was found essential for proliferation in BC cell lines with 3q26 amplification [16]. In a multi-tumor tissue microarray (TMA), including 36 cases of ductal breast cancer, *SEC62* showed over-expression in 32 cases (89%), whereas no *SEC62* overexpression was shown in the normal tissue samples (0%) [12]. Based on these promising results, we investigated *SEC62* expression in 53 cases of breast cancer with immunohistochemistry to evaluate its possible role as a prognostic and predictive biomarker in this tumor entity.

Methods

Patients and tissue samples

Records from all the patients treated with mammary carcinoma in the Department of Obstetrics and Gynecology and at the University of Saarland were reviewed. Inclusion criteria were: patient consent, formalin-fixed, paraffin-embedded tissue sample in our tumor bank and complete clinical follow-up until at least 1st January 2017 ($n=71$). All non-ductal cases ($n=16$) were excluded. Due to insufficient slide quality and no residual material to repeat the staining, another two cases were excluded. The remaining 53 cases with invasive ductal mammary carcinoma were included in the analysis. Clinicopathologic subtyping according to St. Gallen Consensus 2013 was performed according to immunohistological criteria which were determined during routine diagnosis [5]. In the case of Luminal A tumors, all criteria had to be met: estrogen receptor (ER) and progesterone receptor (PgR) positive, Her2neu negative, Ki67 "low" (defined as < 15%) [17]. In the Luminal B-group, one of the following criteria had to be met in addition to ER positivity and Her2neu negativity: PgR negative or PgR "low" (PgR low was defined as PgR expression < 20%); Ki67 "high" (defined as ≥ 15%) [8]. All ER- and Her2neu-positive tumors were categorized as Luminal B. Her2neu-positive (non-luminal) tumors were defined as Her2neu positivity and hormone-receptor negativity. Hormone-receptor-negative, Her2neu-negative tumors were categorized as "triple negative".

All patients provided written consent for the use of their tissue samples and the study protocol was approved by the local ethics board.

Immunohistochemical (IHC) analysis of SEC62 protein level

Tissue samples from each of 53 BC cases were previously HE stained and evaluated by a pathologist. Representative formalin-fixed paraffin-embedded (FFPE) blocks of the primary tumor specimens and histologically tumor-free breast tissue were available from all cases. Sections (3 µm) were transferred onto Superfrost Ultra Plus Microscope Slides (Menzel-Gläser, Braunschweig, Germany) and dried in the incubator at 37 °C overnight. After deparaffinization, heat-induced epitope retrieval was performed in target retrieval solution (Dako S1699, Agilent Technologies, Santa Clara, California) and nonspecific protein binding sites were blocked by incubation in 3% bovine serum albumin (BSA)–phosphate buffer solution (PBS) (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) for 30 min at room temperature. Subsequently, primary antibody incubation was performed with a 1:800 solution (diluted in 1% BSA–PBS) of a specific SEC62 affinity-purified polyclonal rabbit anti-peptide antibody directed against the C terminus of human SEC62 (self-made) for 1 h at room temperature. Each staining series included a positive control and a negative control (without primary antibody). Visualization was performed using the Dako Real Detection System (Agilent Technologies Santa Clara, California) according to the manufacturer's instructions, and slides were counterstained with hematoxylin.

SEC62 immunoreactivity was co-evaluated independently by a pathologist, using the immunoreactive score (IRS) according to Remmele and Stegner as a well-established and unbiased semiquantitative validation system [18]. In this system, staining intensity is classified as no staining (0), weak (1), intermediate (2), or strong (3) staining. The number of stained cells is classified as no stained cells (0), below 10% (1), 10–50% (2), 51–80% (3), or more than 80% (4). The product of the scores for staining intensity and number of stained cells is defined as the immunoreactive score (IRS). For the assessment of the SEC62 protein content of the tumor cells, we rated SEC62 "low" for a score of 0–8, and "high" for 9–12

as described in earlier studies (Fig. 1) [10, 13]. *SEC62* expression in the tumor tissue was compared with the histologically tumor-free tissue of the same patient.

Statistical analysis

OS was calculated as the time from diagnosis date to mortality date or the final documented follow-up date. Survival curves were calculated by the Kaplan–Meier method and compared by the log-rank test. Dichotomized variables were compared (clinicopathological characteristics and SEC62 IHC) with chi square and normally distributed variable (age) were compared with the *t* test. For statistical analysis SPSS software, version 25 (IBM, Chicago, Illinois) was used. $p < 0.05$ (two sided) was considered statistically significant ($\alpha = 0.05$).

Results

SEC62 expression in breast cancer and correlation with clinicopathological characteristics

This analysis included a total of 53 cases with available IHC results and complete intra-institutional follow-up. The mean age at diagnosis was 60 years (range 33–86 years). Of the 53 patients, all (100%) breast cancer cases were identified as expressing *SEC62* (Table 1). In all slides, malignant cells showed cytoplasmic positivity of SEC62 immunostaining, but none of the normal breast tissues stained positively. Since *SEC62* is also highly expressed in lymphocytes, the assessment of cell morphology is, however, indispensable for the evaluation of SEC62 IRS. In mucus and in some specimen artifacts (folds), an intensified staining also occurred, but understanding these phenomena makes it possible to distinguish these artifacts from true positivity. We observed high SEC62-reactivity (defined by IRS > 8) in 31 (58.5%) of 53 cases. We found no correlation between SEC62 IRS and menopausal status, side, tumor size (TNM), UICC (Union Internationale Contre le Cancer) stages [19], lymph node metastasis, hormone-receptor expression, Her2 expression, Ki67 expression, histological grade and adjuvant chemotherapy. High SEC62 IRS was associated with a higher frequency of distant metastasis ($p = 0.024$), but not with metastasis at the time of diagnosis ($p = 0.943$). The groups of patients with low and high SEC62 IRS showed no significant correlation to different prognostic subtypes of breast cancers ($p = 0.740$) (Table 1).

Characteristics of patients with high SEC62 expression levels

We observed that some cases, while most cells showed weak staining, some cells or cell groups showed strong SEC62 staining intensity. Since high *SEC62* expression has already been linked to the migration potential of BC cells, we have examined whether there is a correlation between isolated cells with *SEC62* overexpression and distant metastasis. Focusing only on tumor cells with high intensity immunostaining, independently from the IRS, we found that only one specimen (6.7%) from 15 patients with distant metastases showed no tumor cells with high intensity staining ($p = 0.005$). No further differences in the clinicopathological parameters could be found in comparison of patients with or without tumor cells with high intensity of SEC62 immunostaining (Table 1, Fig. 2).

Impact of SEC62 expression on survival of breast cancer patients

When analyzing the overall survival (OS) rate, the follow-up ranged from 17 to 125 months with a median of 57 months (95% CI 54.33–67.29). The OS rate was significantly lower among patients who had breast cancer with high SEC62 IRS compared to patients with SEC62 IRS ≤ 8 ($p = 0.011$; Table 2, Fig. 3). Patients with tumor cells showing strong SEC62 staining intensity (independently from IRS) had a worse OS rate than patients whose tumors did not contain cells with high-intensity SEC62 staining ($p = 0.024$; Table 2, Fig. 3).

Discussion

In our study, we analyzed *SEC62* expression in 53 BC patients and found a positive *SEC62* expression in all cases. High *SEC62* expression with an IRS > 8, or isolated cells with strong SEC62 staining—independent from IRS—resulted in a poor prognosis. In all invasive ductal breast cancer cases, we found higher *SEC62* expression in tumors in comparison to healthy breast tissue. These results are in line with findings of a previous report, in which *SEC62* overexpression has been observed in 89% of BC cases [12]. In accordance with our results in BC patients, other studies found a significant correlation between high *SEC62* expression and poor clinical outcome in lung cancer [10], head and neck cancer [13, 14], hepatocellular carcinoma [20], and prostate cancer [11].

Data on differences in chemotherapy sensitivity and differences in gene expression suggest that there are significant differences between BC subtypes [21]. These differences can also be observed in the metastasis pattern [22]. In our collective, however, *SEC62* expression was not associated with a particular subtype of BC.

In in vitro assays, SEC62 has been reported to participate in the anchorage-independent growth and migration of breast cancer cell lines [16]. This result is in accordance

Table 1 Characteristics of patients with invasive breast cancer according to SEC62 IRS and impact of cells with high SEC62 immunostaining intensity grade

Characteristics	No. of patients $N=53$	SEC62 IRS		p	Maximum SEC62 staining intensity		p
		SEC62 low (IRS ≤ 8) $N=22$ (41.5%)	SEC62 high (IRS > 8) $N=31$ (58.5%)		Low–moderate (intensity grade 1–2) $N=15$ (28.3%)	High (intensity grade 3) $N=38$ (71.7%)	
Age (mean, ±SD)		59.5 ± 11.8	60.7 ± 14.5	0.745[a]	61.2 ± 12.7	59.8 ± 13.7	0.734[a]

Characteristics	No. of patients $N=53$		SEC62 IRS				p	Maximum SEC62 staining intensity				p
			SEC62 low (IRS ≤ 8) $N=22$ (41.5%)		SEC62 high (IRS > 8) $N=31$ (58.5%)			Low–moderate (intensity grade 1–2) $N=15$ (28.3%)		High (intensity grade 3) $N=38$ (71.7%)		
	n	%	n	%	n	%		n	%	n	%	
Menopausal status												
Premenopausal	12	22.6	5	22.7	7	22.6	0.990[b]	3	20.0	9	23.7	0.773[b]
Postmenopausal	41	77.4	17	77.3	24	77.4		12	80.0	29	76.3	
Side												
Left	31	58.5	12	54.5	19	61.3	0.623[b]	7	46.7	24	63.2	0.272[b]
Right	22	41.5	10	45.5	12	38.7		8	53.3	14	36.8	
Tumor size (TNM)												
T1	27	50.9	10	45.5	17	54.8	0.603[b]	5	33.3	22	57.9	0.330[b]
T2	22	41.5	10	45.5	12	38.8		9	60.0	13	34.2	
T3	1	1.9	0	0	1	3.2		0	0	1	2.6	
T4	3	5.7	2	9	1	3.2		1	6.7	2	5.3	
UICC stage												
I	19	35.8	7	31.8	12	38.7	0.689[b]	3	20.0	16	42.1	0.106[b]
II	23	43.4	11	50.0	12	38.7		10	66.7	13	34.2	
III	6	11.3	2	9.1	4	12.9		2	13.3	4	10.5	
IV	5	9.5	2	9.1	3	9.7		0	0	5	13.2	
Lymph node involvement												
No	27	50.9	12	54.5	15	48.4	0.659[b]	9	60.0	18	47.4	0.407[b]
Yes	26	49.1	10	45.5	16	51.6		6	40.0	20	52.6	
Distant metastasis												
No	34	64.4	18	81.8	16	51.6	0.024[b]	14	93.3	20	52.6	0.005[b]
Yes	19	35.8	4	18.2	15	48.4		1	6.7	18	47.4	
Metastasis at time of diagnosis												
No	48	90.6	20	90.9	28	90.3	0.943[b]	15	100	33	86.8	0.140[b]
Yes	5	9.4	2	9.1	3	9.7		0	0	5	13.2	

Table 1 (continued)

Characteristics	No. of patients $N=53$		SEC62 IRS				p	Maximum SEC62 staining intensity				p
			SEC62 low (IRS ≤ 8) $N=22$ (41.5%)		SEC62 high (IRS > 8) $N=31$ (58.5%)			Low–moderate (intensity grade 1–2) $N=15$ (28.3%)		High (intensity grade 3) $N=38$ (71.7%)		
	n	%	*n*	%	*n*	%		*n*	%	*n*	%	
Estrogen receptor												
Negative	13	24.5	4	18.2	9	29.0	0.366[b]	3	20.0	10	26.3	0.630[b]
Positive	40	75.5	18	80.8	22	71.0		12	80.0	28	73.7	
Progesterone receptor												
Negative	19	35.8	7	31.8	12	38.7	0.606[b]	6	40.0	13	34.2	0.692[b]
Positive	34	64.2	15	68.2	19	61.3		9	60.0	25	65.8	
HER2												
Negative	46	86.8	20	90.9	26	83.9	0.456[b]	14	93.3	32	84.2	0.377[b]
Positive	7	13.2	2	9.1	5	16.1		1	6.7	6	15.8	
Histological Grade												
G1	4	7.5	2	9.1	2	6.5	0.731[b]	0	0	4	10.5	0.392[b]
G2	34	64.2	15	68.2	19	61.4		11	73.3	23	60.6	
G3	15	28.3	5	22.7	10	32.2		4	26.7	11	28.9	
Ki67 Expression												
< 15%	24	45.3	10	45.5	14	45.2	0.983[b]	8	53.3	16	42.1	0.459[b]
≥ 15%	29	54.7	12	54.5	17	54.7		7	46.7	22	57.9	
Subtype												
Luminal A	21	39.6	9	40.9	12	38.7	0.740[b]	7	46.7	14	36.8	0.874[b]
Luminal B HER2 negative	14	26.4	7	31.8	7	22.6		4	26.6	10	26.3	
Luminal B HER2 positive	5	9.4	2	9.1	3	9.6		1	6.7	4	10.5	
Non Luminal	2	3.8	0	0	2	6.5		0	0	2	5.3	
Triple negative	11	20.8	4	18.2	7	22.6		3	20	8	21.1	
Adjuvant chemotherapy												
No	12	22.6	5	22.7	7	22.6	0.990[b]	4	26.7	11	21.1	0.660[b]
Yes	41	77.4	17	77.3	24	77.4		8	73.3	30	78.9	

UICC Union Internationale Contre le Cancer, *IRS* immunoreactive score

[a] *t* test

[b] Chi-square test

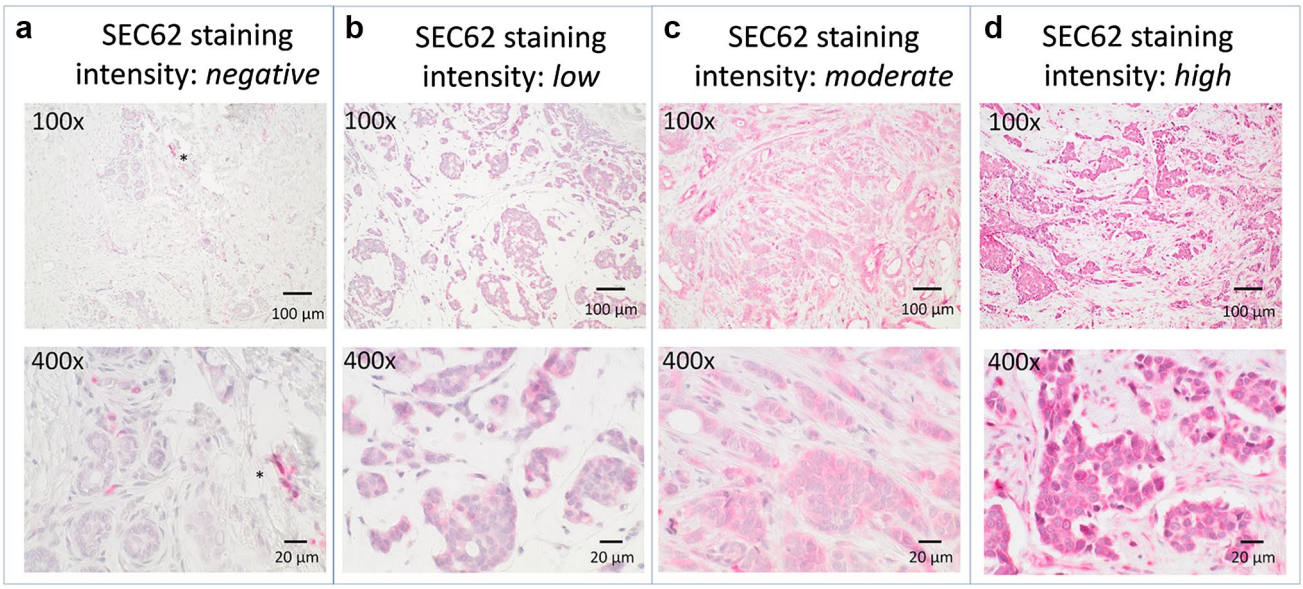

Fig. 1 SEC62 IHC. **a** Negative *SEC62* expression in normal breast tissue, as well as **b** low, **c** moderate, and **d** high immunostaining intensity in breast cancer. *SEC62* expression is indicated by a red signal, counterstaining with hematoxylin (blue). *Unspecific staining of lymphocytes

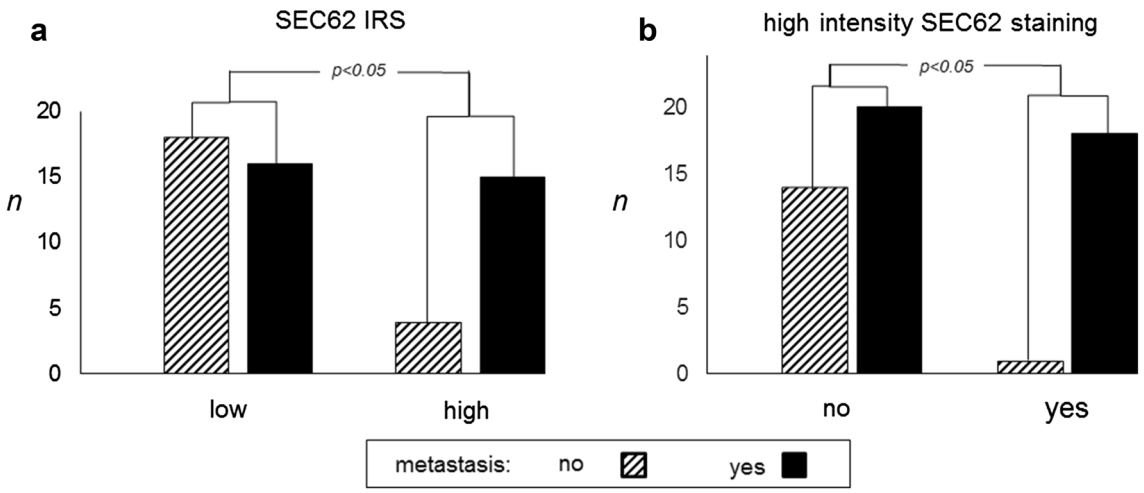

Fig. 2 Impact of *SEC62* expression level in the tumor cells on metastasis. Tumors with **a** high SEC62 IRS or **b** contain cells with high intensity SEC62 staining have frequent distant metastases

Table 2 Summary of immunohistochemical expression analysis and influence on survival time in head and neck squamous cell carcinoma patients

SEC62 IHC	*N*	Survival *n* (%)	Mean time (months)	95% CI		*p* value
				Min.	Max.	
IRS > 8	31	17 (54.8)	72.39	60.83	83.96	0.011
IRS ≤ 8	22	18 (81.8%)	107.02	123.57	83.95	
Tumor cells with high intensity staining						
Yes	38	21 (55.3)	82.99	68.83	97.17	0.024
No	15	14 (93.3)	83.85	77.91	89.79	

IHC immunohistochemistry, *IRS* immunoreactive score, *CI* confidence interval

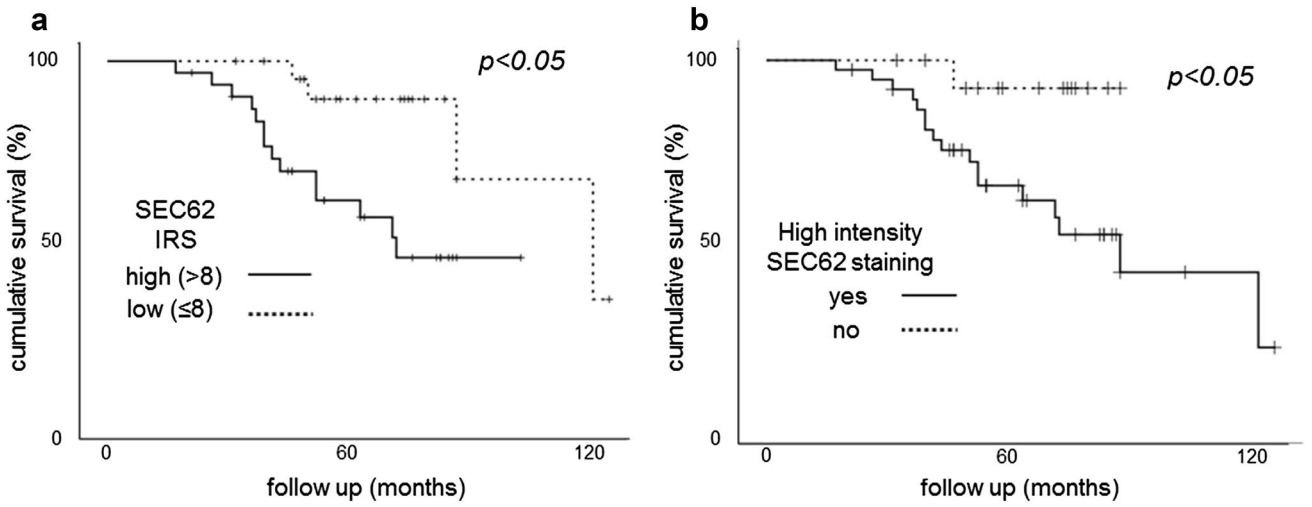

Fig. 3 Overall survival according to **a** SEC62 IRS and **b** presence of tumor cells with high intensity SEC62 staining. Overexpression of SEC62 generally (IRS > 8) or in even a smaller tumor area, independently from IRS predicted a poorer overall survival in invasive ductal breast cancer patients ($p = 0.011$ and $p = 0.024$, respectively)

with our finding of higher SEC62 immunostaining in breast cancer patients with distant metastases (Table 2) and points towards the potential role of SEC62 in tumor cell migration and metastasis. Previous studies have demonstrated the role of the *SEC62* gene in cell migration in cell culture experiments for several tumor cell lines. However, it is not yet clear how this function of SEC62 is mediated at the molecular level [10, 12, 23].

Only one specimen from 15 (6.7%) patients with distant metastases showed no tumor cells with high-intensity staining (Table 2). Hence, we hypothesize that cells with *SEC62* overexpression have the potential to form metastases. In human BC, tumor cells have been shown to disseminate earlier than previous sequential models of cancer development have suggested. These cells often show fewer or different genetic aberrations than the primary tumor, which shows that the dissemination does not start with the development of a highly developed tumor cell [24]. This observation is also reflected in our results. Alone, the presence of single tumor cells with a high *SEC62* expression increases the risk of metastasis and thus worsens the prognosis even at lower IRS. In contrast, if no SEC62 high-intensity cells are found, this could indicate a very low risk of metastasis for the patients.

As SEC62 is involved in the protein translocation process of the endoplasmic reticulum, we speculate that SEC62 may regulate the expression of migration-relevant proteins. To date, however, no corresponding proteins have been identified. Linxweiler et al. could not show any influence of SEC62 on the expression of other EMT markers, such as vimentin and E-cadherin [23]. *SEC62* over-expression has an inhibitory effect on the SEC61-mediated calcium efflux from the endoplasmic reticulum lumen [23]. SEC62's influence on the intracellular Ca^{2+} homeostasis could, therefore, also be a possible connection between SEC62 and cell migration [25]. However, the exact link in the molecular mechanisms between SEC62 and the Ca^{2+} efflux, as well as Ca^{2+} and cellular migration are also not fully understood [26].

SEC62 could also serve as a therapeutic target for the treatment of BC patients. An RNA interference-based depletion of SEC62 protein has inhibited tumor cell migration in in vitro studies [10, 23]. A reduction in the SEC62 content of tumor cells could impair their ability to migrate and metastasize. In addition to a direct inhibition of gene expression, it is also possible to antagonize the function of SEC62 in cellular calcium homeostasis with calmodulin (CaM) antagonists, which has been shown in cervical adenocarcinoma (HeLa) and prostate cancer (PC3) cell lines [25]. CaM antagonists trifluoperazine (TFP) and Ophiobolin A induced a dose-dependent inhibition of tumor cell migration in cervical adenocarcinoma (HeLa), lung cancer (H1299, A549, and BC01), thyroid cancer (BHT101 and ML1), and prostate cancer (PC3) cell lines, and an additional inhibition of tumor cell proliferation at higher concentrations, i.e., the same effects reported for SEC62 depleted tumor cells [10, 12, 13, 23, 25]. TFP has been used for the treatment of patients with psychiatric disorders for many years. A retrospective study has shown favorable outcomes, including delayed tumor progression and prolonged survival of cancer patients with chronic TFP treatment [27].

In addition to an increased potential for migration, *SEC62* overexpressing cancer cells show a higher tolerance to cellular stress, such as thapsigargin (TG)-induced ER stress [23, 25]. Thapsigargin, a selective, noncompetitive inhibitor of sarcoendoplasmic reticulum Ca^{2+}-ATPase

(SERCA), reduces the proliferation and migration of several tumor cell types, including BC. The ability of thapsigargin to induce apoptosis in BC cell lines has also been demonstrated in vitro [28]. In targeted antitumor prodrug therapy strategies, a drug can be delivered to the tumor cells in a dose-intensive and selective manner, maximizing efficacy and minimizing toxicity. This generally cytotoxic substance has been clinically tested in such therapy approaches in the therapy for PSMA-expressing (prostate-specific membrane antigen) solid tumors [29]. PSMA expression of tumor-associated microvasculature was observed in 74% of primary BC specimens and 100% in brain metastases. According to this data, this therapy may be a promising option in the future, especially for patients with brain metastases [29, 30].

In particular, tumor patients with low *SEC62* expression might benefit from cancer treatment with thapsigargin or analogous agents, however, tumor cells with high *SEC62* expression might be resistant to this therapy [10, 12, 15, 31–33]. However, with the inhibition of SEC62, increased sensitivity to chemotherapy could potentially be achieved in this group of patients.

Limitation of the study is the small number of patients. Since the IRS was assessed by a single investigator, the interobserver variability was not evaluated. However, interobserver variability is a known source of error in immunohistochemical studies. Further studies including more investigators, as well as a large number of patients should be performed to confirm the results of this preliminary study.

Conclusions

Our results suggest that *SEC62* expression may serve as a prognostic marker for patients with breast cancer.

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Author contribution FZT project development, data collection and analysis, manuscript writing. JCR review and editing. ML data analysis, review and editing. MK data collection, review and editing. RMB project development, data collection, review and editing. FB review and editing, visualization. CU data collection, review and editing. EFS review and editing. BS review and editing. IJB review and editing.

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Compliance with ethical standards

Conflict of interest We declare that we have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All patients provided written consent for the use of their tissue samples. The study was approved by the Ethics Committee of Saarland (No: 183/17).

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